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(57) Abstract

Bi-specific antibody molecules having a first binding affinity for a T-cell-receptor capable of activating killing and a second binding affinity for immunoglobulin, and fragments thereof retaining the binding affinity of the whole molecule. are of value in treating disease, particularly neoplastic. viral and parasitic diseases. The cytotoxic molecules or fragments are targeted against selected target cells through use in vivo in conjunction with antibody molecules or fragments thereof having a binding affinity for the target cells. Alternatively. the bi-specific antibody molecules or fragments may be combined in vitro with such antibody molecules or fragments having a binding affinity for target cells. to provide a bi-specific antibody conjugate or fragment thereof having the binding affinities of its two components which may be used in vivo in treating disease.

ANTIBODIES

This invention relates to novel forms of antibody and their use as targeted cytotoxic agents, particularly in the treatment of neoplastic disease.

The treatment of neoplastic disease still remains an
05 intractable problem despite the fact that a very wide range of cytotoxic agents has now been developed for use in the treatment of the disease. One recent approach utilises the phenomenon of effector cell retargeting (ECR) to destroy tumour cells. In this approach a bi-specific antibody is constructed which has
10 both an anti-T cell and an anti-tumour antigen activity. In the first application of this technique (Staerz et al, Nature, 1985, 314, 628, Perez et al, Nature, 1985, 316, 354 and Liu et al, Proceedings of the National Academy of Sciences of the USA, 1985, 8648) a bivalent antibody conjugate was constructed by
15 chemical means but in a further application of the technique (Staerz and Bevan, Proceedings of the National Academy of Sciences of the USA, 1986, 83, 1453 and Immunology Today, 1986, 7, 241) hybridoma technology has been employed to produce a bi-specific antibody molecule. The bi-specific antibody exerts
20 its effect by binding both to a tumour cell or other form of target cell, such as a virally infected cell, and to a T-cell thereby effecting destruction of the former by the action of the latter.

The use of effector cell retargeting as described in the art
25 does however have various inherent disadvantages. In particular, the bi-specific antibody will in general utilise to any significant extent only one of the natural cell killing mechanisms of which the body is capable in order to kill the tumour cells. It is an object of the present invention to
30 provide an improved method for the destruction of tumour cells utilising the natural killing mechanisms of the body including the use of effector cell retargeting.

Accordingly the present invention comprises a bi-specific
antibody molecule consisting of two heavy chains and two light
chains and having a first binding affinity for a T-cell receptor
capable of activating killing and a second binding affinity for

05 immunoglobulin, which immunoglobulin binding affinity is not one
which causes the bi-specific antibody molecule to be directly
targeted to cells present in the species in which the T-cell
receptor occurs, or a fragment thereof retaining the binding
affinity of the whole molecule.

10 It will be appreciated that the bi-specific antibody
molecules of the present invention differ from those bi-specific
antibodies of the 1985 Staerz et al, Perez et al and Liu et al
papers in as far as, although being bi-specific, the present
antibodies have the normal form of an antibody molecule in which

15 two light chains and two heavy chains are present. By way of
contrast the bi-specific antibodies described in 1985 were
conjugates produced by the chemical cross linking of two normal
antibody molecules and contained four light chains and four
heavy chains. Although the bi-specific antibodies described in

20 the 1986 Staerz and Bevan papers consist of antibody molecules
rather than conjugates, the binding affinities of these
molecules are of course different from those of the present
invention and, moreover, the authors failed to appreciate the
problems inherent in the use of their molecules as regards the

25 killing of T-cells by the molecules. The present invention is
further directed to•the mitigation of this previously quite
unrecognised problem. The 1985 Liu et al paper describes an
antibody conjugate having one binding affinity for the human T3
complex and a second binding affinity for the idiotype of the

30 surface immunoglobulin of a human B-lymphoma. Such an idiotype
represents a true tumour specific transplantation antigen and is
used to direct the antibody conjugate to tumour cells. The
Liu et al antibody conjugate therefore essentially has
anti-T-cell and anti-tumour cell affinities and, apart from its

35 conjugate nature, may be distinguished from the bi-specific

antibody molecules of the present invention which function
against T-cells through binding with a further antibody having
an anti-tumour cell, or other target cell affinity.

In their primary use the bi-specific antibody molecules
05   described above are administered in conjunction with a separate
antibody having a binding affinity for target cells. Such a
procedure enables various of the body's cell killing mechanisms
to be brought into play. Firstly, the antibody having a binding
affinity for target cells can bind to a target cell and effect
10   killing thereof through an Fc receptor mediated killing
mechanism, for example via antibody-dependent cell-mediated
cytotoxicity (ADCC) involving K cells, neutrophils and
macrophages, via phagocytosis by macrophages and cells of the
reticuloendothelial system, or via complement activation.
15   Secondly, the resultant target cell/antibody system can bind
further with the bi-specific antibody molecule through the
immunoglobulin affinity of this molecule, the whole system then
binding with a T-cell through the T-cell affinity of the
bi-specific antibody molecule and utilising T-cell toxicity to
20   destroy the target cell.

The bi-specific antibody molecules of the present invention
function by binding to T-cells in order to direct their toxicity
and the T-cell binding affinity of the antibody molecule may be
specific for any T-cell receptor which will cause killing. Thus
25   the receptor may be associated with the ability of the T-cells
to kill either directly or indirectly through the assistance of
other cell types or any other agent. Thus, the receptor may be
one which is associated with the ability of cytotoxic T-cells to
kill directly or with the ability of helper T-cells to assist
30   killing by B-cells, or with any other indirect mode of killing
(natural or artificial). Although receptors capable of
activating direct killing are of particular interest, it will be
appreciated that many receptors are associated with both direct
and indirect modes of killing.

In particular, the binding affinity may be directed against one or both of the α and β chains which comprise the T-cell antigen specific receptor termed Ti, and which are present on the vast majority of T-cells, or it may be directed against the receptor-associated CD3 unit (previously identified as T3) as a whole or against one of the individual chains thereof. Thus, studies with human cells presently indicate that the T-cell receptor exists as a complex of two chains identified as α (M_r about 50,000) and β (M_r about 40.000) which are coded for by genes which are somatically rearranged in an analogous fashion to immunoglobulin genes. Each T-cell therefore possesses a unique rearrangement of genes coding for these two chains. These two chains are found in association with at least three other chains which comprise the CD3 complex and are identified as γ (M_r about 25,000), δ (M_r about 20,000) and ϵ (M_r about 20,000).

Antibodies having a T-cell binding affinity, either for a known or a novel receptor, may be identified by an assay procedure which we have developed. Most hybridomas as well as secreting antibody have small amounts of cell surface antibody. Thus, for example, a mouse hybridoma making antibody against rat IgG2b is capable of trapping a rat IgG2b antibody by virtue of the small amount of antibody against rat IgG2b on its surface. If the hybridoma cells are labelled with a radioactive label, for example ^{51}Cr, and then incubated with a mixture of T-cells and monoclonal rat IgG2b antibodies against T-cells, the hybridoma cells will bind to the antibody which will in turn bind to the T-cells, thereby leading to killing of the hybridoma and consequent release of the radioactivity. Such a procedure therefore provides a means of detecting rat IgG2b antibodies with an appropriate T-cell binding capacity (i.e. those capable of inducing killing). By selection of an appropriate form of hybridoma such a screen can be conducted among antibodies of any species and class or subclass. The hybridomas producing antibodies detected in such an assay procedure may be used in preparing the bi-specific antibodies of the present invention by techniques described hereinafter.

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Anti-non human T-cell/anti-immunoglobulin bi-specific
antibody molecules are of interest, particularly in a research
context, for example as a model system in the investigation of
the important requirements for cell killing with animal tumours
05   such as those of the rat and mouse. However, the major area of
interest of the present invention is in human medicine and
bi-specific antibodies having a first binding affinity directed
against human T-cells are therefore of particular interest,
these most usually being used in conjunction with a separate
10   antibody having a binding affinity for target cells present in
the human body. Using assay procedures such as that described
above it is possible to select from among hybridomas producing
monoclonal antibodies having the ability to bind with human
T-cells those which are capable of triggering T-cell
15   cytotoxicity, one example of such an antibody being the rat
IgG2b antibody produced by the hybridoma YTH 12.5.14.2 (and all
related subclones) which is described in Example 2 hereinafter.
This antibody binds to the T-cell antigen CD3 but other
antibodies of different specificities may also be similarly
20   selected. Other examples include the anti-human CD2 rat IgG2b
antibodies produced by the hybridoma YTH 655(5)6 and
YTH 616.7.10 (H.P. Tighe, Ph.D. Thesis entitled "Monoclonal
antibodies against cell surface antigens involved in leukocyte
function", University of Cambridge, 1987) and the anti-human CD3
25   mouse IgG2a antibodies produced by the hybridoma OKT3 which is
described in Example 3, and also the group of anti-CD3 mouse Ig
antibodies listed by Kurrle et al, Leukocyte Typing II,
Volume 1, Human T-Lymphocytes, edited by Reinherz et al,
Springer Verlag, 1985, page 137, and various of the antibodies
30   listed for the Third International Workshop and Conference on
Human Leukocyte Differentiation Antigens, Leukocyte Typing III,
White Cell Differentiation Antigens, edited by McMichael, Oxford
University Press, 1987, in particular those antibodies against
the CD3 unit and also against the Ti receptor, as well as
35   certain of the anti-CD2 antibodies. It will be appreciated that
hybridomas producing all of these antibodies may of course be

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used in derivative forms (as reclones or subclones) or that
analogous hybridomas of similar specificity may be employed.

The second binding affinity of the bi-specific antibody
molecules according to the present invention may be directed
05   against any of the variety cf binding sites present in antibody
immunoglobulins, being for example anti-allotype, anti-isotype
(particularly -subclass), anti-species or to a lesser degree of
preference, anti-idiotype, in its specificity. (The word
isotype is used in the art to designate a particular class
10   and/or subclass so that in the rat, for example, IgM, IgG1,
IgG2a, IgG2b and IgG2c each constitute a separate isotype. In
the present instance an anti-isotype binding affinity is most
usually anti-subclass, for example anti-IgG1 etc. rather than
anti-IgG, although it is possible for the affinity to be
15   anti-class particularly when, as in the case of IgM, no
subclasses exist.) In addition to such forms of binding
affinity for immunoglobulins it will be appreciated that the
immunoglobulin affinity of the bi-specific antibody molecule may
also be directed against a site in the immunoglobulin exposed by
20   the formation of a fragment thereof, for example the F(ab')$_2$
fragment of the anti-target cell antibody as described
hereinafter. Alternatively the affinity may be for an
artificially created site on the immunoglobulin produced by the
attachment of a hapten thereto. Such an affinity for
25   immunoglobulin based upon anti-hapten binding may be utilised,
for example, with fluorescein isothiocyanate labelled or
biotinylated immunoglobulins.

The primary use of the bi-specific antibody molecules of the
present invention is in indirect ECR where binding of the
30   molecules to target cells is effected through a separate
antibody molecule having a binding affinity for target cells.
Accordingly, the immunoglobulin binding affinity is not one
which causes the bi-specific antibody molecule to be targeted to
cells, in particular tumour cells, without the use of a separate
35   anti-target cell antibody. The immunoglobulin therefore is not

one which functions as a cell surface antigen, and particularly is not an antigen present on the surface of tumour cells. In particular the immunoglobulin specifity of the bi-specific antibody molecule excludes any specificity for an endogenously
05   synthesised and expressed cell surface immunoglobulin, particularly one which appears on the surface of tumour cells. An anti-idiotype affinity is an especial case of a less favoured specificity, certain forms of anti-idiotype binding affinity being excluded in view of the functioning of many idiotypes as
10   cell surface antigens and other forms, although not excluded, being less preferred than other forms of immunoglobulin binding affinity since an anti-idiotype can block antibody/antigen binding. A specific example of an excluded specificity involving a cell surface immunoglobulin and idiotype is the
15   idiotype of the surface immunoglobulin of a human B-lymphoma, such as that described by Liu et al, ibid, which functions as a tumour specific cell surface antigen.

It will be appreciated, however, that for maximum freedom in selection of the anti-target cell antibodies which are employed
20   in conjunction with the bi-specific antibodies, the immunoglobulin affinity of the latter is preferably as non-specific as possible. This preference is however subject to the proviso that the bi-specific antibody molecule is intended for use in vivo and that any undesirable side reaction with the
25   patient's body should be avoided in as far as possible. In practice, the simplest way of avoiding undesired reactions between the bi-specific antibody molecule and immunoglobulin molecules naturally present in the patient's body is for the immunoglobulin binding affinity to relate to a species other
30   than that to which the T-cell receptor binding affinity relates. Commonly the latter species is man and the former may conveniently be another mammalian species, for example the rabbit and particularly the mouse or especially the rat. Thus, it is convenient if, for example, the bi-specific antibody will
35   bind with any of the various classes of antibody as discussed

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hereinafter and particularly with both IgM antibodies and IgG
antibodies, including the various subclasses of the latter.
Moreover, it is advantageous if the binding affinity is not
totally species specific so that, for example, binding to at
05   least both of rat and mouse antibodies is possible.

In practical terms, however, this may be too demanding a
requirement and it may therefore be appropriate to use a small
group of anti-immunoglobulin antibodies specific for the rat or
mouse and possibly even for different subclasses thereof.

10   It should be appreciated that an important preference for
the anti-immunoglobulin specificity is that it does not cause
auto-reactivity. Thus, for example, if the bi-specific antibody
molecule is itself composed of rat IgG2b heavy chains and rat
kappa light chains, the anti-immunoglobulin specificity should

15   exclude anti-rat IgG2b and anti-rat kappa. The bi-specific
antibody molecule described in Example 2 hereinafter is a hybrid
between the antibody YTH 12.5.14.2 (a rat IgG2b with a lambda
light chain which is specific for the CD3 antigen) and the
antibody RG11/15.5 (a mouse IgG2a with a kappa light chain which

20   is specific for the rat kappa 1b allotype). This combination,
which avoids auto-reactivity, can be used in conjunction with
any rat antibody containing a rat kappa 1b allotype light chain.

As regards the anti-target cell antibodies used in
conjunction with the bi-specific antibodies of the present

25   invention, these may have a binding affinity for any antigen
present on the surface of a target cell. The target cell may be
any cell which may be beneficially removed from the body.
Examples include virally infected cells (viruses themselves not
normally being attacked by T-cells), the virus being of various

30   types including the influenza and rabies viruses, and both
parasitized cells and parasites themselves including those
responsible for malaria, leprosy, trypanosomiasis and
schistomiasis, as well as tapeworms and other parasitic worms
such as helminths. The preferred target cells are however

35   tumour cells, the anti-target cell antibodies having a binding

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affinity for any tumour-associated antigen. The ideal situation would be for the affinity to be for a tumour-specific antigen, i.e. an antigen found on tumour cells only and not on normal cells. However, the B-cell Ig idiotype which has been employed
05 in the treatment of B-cell malignancies such as BCLL is one of the very few examples of such antigens which exist and in practice the tumour-associated antigen will also exist on normal cells. Preferably, the antigen is anomalously expressed at higher levels or in an appropriate way on tumour cells thereby
10 allowing an enhanced level of antibody-antigen reaction with the tumour cells but even this is not completely necessary. Thus, for example even in the extreme case where there is no differentiation between tumour cells and normal haemopoietic cells, the toxic effects of the treatment on the normal cells
15 can be countered by the use of marrow transplants or of removal of the patient's own bone marrow prior to the treatment and its return thereafter, conveniently following separate treatment of the bone marrow in vitro for the removal of tumour cells. Examples of anti-tumour antibodies of particular interest are
20 antibodies against antigens which define clusters of differentiation (CD) of the haemopoietic system recognised by groups of monoclonal antibodies standardised and characterised by International Workshops on Human Leukocyte Differentiation Antigens (Paris 1980, Boston 1983, Oxford 1986), and antibodies
25 to the common acute lymphoblastic leukaemia-associated antigen CALLA, the carcinoembryonic antigen as expressed on, human colon carcinoma, and the human melanoma-associated ganglioside GD3. A specific example is the human B-cell differentiation antigen CD19 which is expressed on normal B-cells and many malignant
30 B-cells and B-cell lines.

The anti-target cell antibodies may be produced by classical techniques as a polyclonal preparation but are more conveniently produced as monoclonal antibodies using hybridoma technology, for example as described in US Patent 4,172,124 and in a wide
35 range of scientific papers present in the literature.

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The bi-specific antibody molecules described herein may be produced by the chemical linkage of the halves of two antibodies, which may be produced by classical techniques or by hybridoma technology, one of the first binding activity and

05   another of the second binding activity. However, particularly preferred bi-specific antibody molecules according to the present invention are those produced directly by the techniques of hybridoma technology modified as necessary for the production of bi- rather than mono-specific antibodies. Such techniques

10   for the preparation of bi-specific antibodies, in general, are described in European Patent Application 0068763 and PCT Application WO 83/03679 which relate broadly to bi-specific antibody molecules produced by hybridoma technology. Examples of myeloma starting materials for use both in the preparation of

15   the bi-specific antibodies and also the antitarget cell antibodies are the Y3-Ag 1.2.3 myeloma of European Patent No. 001459 (C.N.C.M. No. I-078), the Y82/3.0.Ag.20 myeloma of European Patent No. 0043718, the myeloma P3-X63-Ag8 (A.T.C.C. No. CRL 1597), the myeloma NS1/1-Ag4-1 (A.T.C.C. No. TIB 18) and

20   the myeloma P3 (Staerz et al, Journal of Immunology, 1985, 134, 3994-4000), as well as the various human myelomas referred to in the literature such as those of European Patent Applications 0062409 and 0148644, UK Patent 2086937 and US Patent 4529694. It is preferred that each hybridoma is either derived from a

25   myeloma which does not express a light chain or is a myeloma light chain loss variant, i.e. being HL rather than HLK. The techniques used in the preparation of the bi-specific antibodies of the present invention closely parallel those described in U.K. Patent Application 2,144,147A which may also be applied in

30   the case of the present invention, although the binding affinities of the fusion partners are of course different. We have, however, developed a variant of these techniques which has proved to be of especial value. As in previous techniques described for the preparation of bi-specific antibody molecules

35   our procedure involves the fusion of two hybridomas, but in the present instance one of these produces antibodies directed

against T-cells and the other produces antibodies directed against immunoglobulin. The two hybridomas may be fused by the conventional procedures, as illustrated in the Examples, to produce a hybridoma secreting bi-specific antibody molecules having each of the specificities of the hybridomas from which they derive. It will be appreciated, however, that one of the most difficult stages of any hybridoma-producing fusion is the selection from the fusion mixture of the desired type of bi-specific hybridoma and the procedure we have developed involves features directed particularly to this end.

In our procedure, the first hybridoma used has a drug selectable marker which may conveniently be an enzyme deficiency, for example of thymidine kinase (TK) or particularly of hypoxanthine-guanine phosphoribosyl transferase (HPRT). Such hybridomas are obtained by selecting cells from a growth medium containing 5-bromouracyl deoxyribose or 2-aminopurine (for TK) or 8-azaguanine or particularly 6-thioguanine (for HPRT). Cells selected for growth on a medium containing the appropriate drug lack the enzyme in question and, in the case of both TK and HPRT, will therefore be incapable of growth in a medium containing hypoxanthine, aminopterin and thymidine (HAT) since the aminopterin blocks the main pathway for purine and pyrimidine synthesis and the lack of HPRT or TK removes the ability possessed by normal cells of utilising the hypoxanthine to make purines and the thymidine to make pyridimines.

The second hybridoma employed in our procedure is poisoned with a lethal dose of an irreversible biochemical inhibitor, for example diethylpyrocarbonate and particularly iodoacetamide. Such an inhibitor poisons the cells but does not damage their DNA, which codes for immunoglobulin expression and also for the HPRT enzyme. Following the treatment of these cells they are washed to remove any excess of the inhibitor and are then ready for use, the overall structure of the cells remaining intact for several hours after the treatment, the fusion typically being carried out within 0.5 to 1 hour.

Fusion of the two hybridomas will produce a fused cell system which possesses the DNA from both hybridomas and in which any short-term loss of vital enzyme function from the poisoned cells will be complemented by the enzymes derived from the other

05 hybridoma. Following the fusion of the two hybridomas the fusion mixture is cultured in a medium free from inhibitors when cells of the unfused hybridoma which has been poisoned will gradually die whilst cells of the other unfused hybridoma and the fused cells will survive. Selection is then commenced with,

10 for example, a HAT-containing medium when cells of the unfused hybridoma lacking TK or HPRT will die but the fused cells will survive since the enzyme deficiency is met by the DNA from the other hybridoma. Iodoacetamide has been found to cause cell death within 1 to 24 hours but it has been found that a greater

15 level of hybridisation is generally obtained if selection is delayed for 2 to 3 days, possibly because HPRT expression derived from the poisoned cell requires a little time to occur fully. Moreover, best results have been obtained using an equal or higher proportion of untreated cells to iodoacetamide-treated

20 cells, for example from 1:1 to 10:1.

The mixture of cells from which both types of unfused hybridoma cell have been eliminated is then treated further in the usual way to isolate therefrom hybridoma secreting monoclonal antibodies having the desired bi-specificity. Such

25 hybridomas may be cultured either in vitro or in vivo by conventional techniques in order to produce supplies of the monoclonal antibodies.

Although the use of the type of bi-specific antibody molecules and fragments thereof described herein of itself

30 represents an improvement over the use of the bi-specific antibodies described in the art, in a preferred aspect of the present invention a further improvement may be obtained. Thus, it is quite possible for a bi-specific antibody to induce killing of the T-cells to which it binds through the natural Fc

35 receptor mediated killing mechanisms referred to hereinbefore. Although no reference is made thereto in the Staerz and Bevan papers described hereinbefore, this is a serious deficiency in

the effector cell retargeting procedure of the prior art since
that procedure kills tumour cells only through T-cell toxicity.
In the case of the present invention, the problem is less
serious as other tumour killing mechanisms are also involved but
05   it still reduces the efficiency of tumour cell killing which is
likely to be achieved. In a preferred aspect of the present
invention, therefore, the problem of T-cell destruction may be
mitigated, and preferably substantially avoided, in one of two
ways.
10   The first of these ways is based on an appreciation that the
problem can be overcome by the use of an appropriate combination
of heavy chains in the bi-specific antibody molecules of the
present invention. The overall structure of an immunoglobulin
is determined by the interactions of the various globular
15   domains of the individual chains with each other. These
interactions consist of covalent bonds involving intra-chain and
inter-chain disulphide bridges as well as non-covalent
interactions involving both protein and carbohydrate groups. In
order to activate the killing mechanisms, complement components
20   and Fc receptors must bind to structures present in the
different antibodies but different species, isotypes and
allotypes of antibody have differences in portions of their
protein sequences although they may have many similarities in
other portions of these sequences. Thus different
25   immunoglobulins will interact differently with complement
components and Fc receptors and, in addition, when hybrid
antibody molecules are made the two heavy chains may differ in
sequence at crucial points for their interaction with each other
and this may influence the properties of different combinations.
30   The present invention thus includes an antibody molecule
consisting of two heavy chains and two light chains and having a
first binding affinity for a T-cell receptor capable of
activating killing and a second binding affinity for
immunoglobulin, which immunoglobulin binding affinity is not one
35   which causes the bi-specific antibody molecule to be directly
targeted to cells present in the species in which the T-cell
receptor occurs, the two heavy chains in the molecule being

selected so that an ability for the antibody molecule to utilise
the natural killing mechanisms of the body to destroy the
T-cells is mitigated.

The immunoglobulins which constitute antibodies may be
05   divided into several classes, the major ones of these being
identified as IgG, IgA, IgM, IgD and IgE, of which some, in
particular IgA and especially IgG, may be further divided into
subclasses. The bivalent antibody molecules according to the
present invention preferably contain two heavy chains of the
10   same class but may be of different subclasses within that class
and may be of the same or a different subclass but relate to
different species, the human, rat and mouse being of most
interest, although immunoglobulins of other species, for example
the rabbit, may be employed. Combinations of rat and mouse and
15   rat and rat immunoglobulins are preferred to the mouse and mouse
combination in terms of the higher level of stability of the
corresponding hybridomas.

As regards the Fc receptor mediated killing mechanisms,
particularly ADCC, we have found that although the nature of the
20   heavy chain providing the T-cell binding affinity is of prime
importance, the nature of the heavy chain providing the
immunoglobulin binding affinity is also of importance. Thus,
certain subclasses of immunoglobulin of a first species will not
interact with the cell mediated effector mechanisms of a second
25   species and with those subclasses which will interact when two
heavy chains of the same species and subclass are present in the
immunoglobulin it is possible to interfere with this interaction
by replacing one of the two heavy chains by an appropriate
selection from another species or subclass. With the complement
30   activation mechanism, the nature of the heavy chain providing
the T-cell binding activity may be a more dominant factor in
determining the level of complement activation which occurs than
it is with ADCC, but the nature of the heavy chain providing the
immunoglobulin binding activity can still exert an important
35   effect as discussed hereinafter.

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In selecting an appropriate combination of heavy chains for
use in a bi-specific antibody molecule according to the present
invention it must be appreciated that the species to which the
two heavy chains relate and the species to which the antibody is
05   to be administered are all of importance. Thus, a particular
heavy chain species subclass combination which does not produce
killing of T-cells in the mouse or rat, for example, may well do
so in the human, which is the species of choice as regards the
T-cell binding affinity of the bi-specific antibodies. As
10 .   indicated hereinbefore, human heavy chains may conveniently be
used for at least one of the heavy chains in the bi-specific
antibody molecules of the present invention. Indeed, a
preferred choice is the use of two human heavy chains of the
same class but of a different subclass. However, the relative
15   lack of availability of human myelomas as compared with mouse
and rat myelomas can pose a problem and in practice these
species may often therefore represent the mammalian species of
choice for the heavy chains.

In the human, the IgG class is divided into the subclasses
20   IgG1, IgG2, IgG3 and IgG4 (the IgA class being divided into the
subclasses IgA1 and IgA2) whilst in the mouse and rat only the
IgG class is divided into subclasses, these being IgG1, IgG2a,
IgG2b and IgG3 in the mouse and IgG1, IgG2a, IgG2b and IgG2c in
the rat (the similarly named subclasses not necessarily having
25   similar properties in the mouse and the rat). In practice each
of the heavy chains is most likely to be of mouse or rat IgA or
IgE, particularly IgM and especially IgG, the commonest
situation being that they are each of an IgG subclass and
especially mouse IgG1, IgG2a or IgG2b, and less commonly IgG3,
30   or rat IgG1, IgG2a or IgG2b, and less commonly IgG2c.

In selecting a heavy chain having T-cell binding activity,
the simplest course is to use a class or subclass. for example
of the rat or mouse. which does not lead to the killing of
T-cells in the species in question via an Fc receptor mediated
35   mechanism. A convenient choice for the avoidance of ADCC

killing, in particular, is a heavy chain of the IgM class but
such heavy chains are often particularly effective at producing
killing through complement activation. In terms of activity and
of common availability a heavy chain of the IgG subclass is
05   therefore an usual choice and for the avoidance of killing
through complement activation the order of preference in the
mouse is IgG1 > IgG3 > IgG2a = IgG2b and in the rat is
IgG1 > IgG2c > IgG2a > IgG2b. As regards the avoidance of
killing through other Fc receptor mediated killing mechanisms,
10   particularly via ADCC involving K-cells, the preference in the
mouse is IgG2b, IgG3 and IgG1 > IgG2a and in the rat IgG1 and
especially IgG2a and IgG2c > IgG2b. We have discovered,
however, that the close interaction between the two heavy chains
in bi-specific antibody molecules, but not in conjugates, is
15   such that even though the heavy chain of T-cell binding activity
is one such as mouse IgG2a or rat IgG2b which will promote
killing, it is possible to counteract this killing through the
selection of a suitable form of heavy chain having
immunoglobulin binding activity, in particular one of a
20   different species or of a different isotype or allotype. Among
the other rat IgG subclasses the preferences for inactivating
the rat IgG2b heavy chain are a rat IgG2a or especially a rat
IgG2c heavy chain. With such active heavy chains as rat IgG2b
or mouse IgG2a combination with an alternative form of heavy
25   chain, either by species (for example rat IgG2b/mouse IgG1) or
by subclass, is indicated since a rat IgG2b/rat IgG2b or mouse
IgG2a/mouse IgG2a combination can generally be presumed to be
effective in promoting killing through the ADCC mechanism and
possibly also through the complement activation mechanism. The
30   use of heavy chains of a different allotype or particularly a
different species or isotype can also be of value when the
anti-T-cell heavy chain may exhibit only an insubstantial level
of effectiveness in causing killing (i.e. being substantially
ineffective), possibly acting at a low level through only one of
35   these two mechanisms as may be the case for example with some

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mouse IgG isotypes. Although similar combinations of isotype
may be used in such instances, the use of a difference of
species or isotype will mitigate even the insubstantial level of
effectiveness and provides a clear indication of suitability for
05   the heavy chain combination. In general, the achievement of the
mitigation of the killing of T-cells may be assessed by a
comparison with such bi-specific antibody molecules in which the
heavy chain having the affinity for target cells is the same
(species, isotype and conveniently allotype) as that having the
10   affinity for T-cells.

   In addition to the use of the information given
hereinbefore, simple test procedures may be used to determine
appropriate combinations of heavy chains for use in the
bi-specific antibodies of the present invention. Thus, if it is
15   proposed to use in a bi-specific antibody molecule the heavy
chain/light chain combination of a particular monoclonal
antibody against T-cells which is itself capable of inducing the
killing of T-cells, then the hybridoma producing this monoclonal
antibody can be fused with a hybridoma producing a specified
20   immunoglobulin type of monoclonal antibody of any irrelevant
binding affinity. The resulting bi-specific antibody can then
be tested to see if the T-cell killing ability of the first
heavy chain/light chain combination is negated by combination
with a heavy chain/light chain combination of the immunoglobulin
25   type in question. Thus, for example, by fusing a hybridoma
producing a rat IgG2b antibody against T-cells with a hybridoma
producing any irrelevant rat IgG2a or IgG2c antibody it can be
tested whether the ability of the IgG2b heavy chain to induce
killing by both the ADCC mechanism and through the complement
30   activation mechanism is retained or not. An alternative
approach is to transfect cloned immunoglobulin genes into a
hybridoma so that the cloned gene is expressed in the hybrid
cell and mixed immunoglobulin molecules produced which can be
assayed for activity in T-cell killing by both the ADCC and
35   complement route.

Although such procedures may be used with advantage to
identify heavy chain combinations of particular value, it will
be appreciated that in general any difference of species or
difference of isotype (i.e. either of class or subclass) is
05   sufficient to mitigate the killing of T-cells by the bi-specific
molecule and that many differences of allotype will also achieve
this result. Other criteria relating to the selection of
combinations of heavy chains are discussed hereinafter.

      Even though an appropriate combination of heavy chains not
10   leading to the killing of T-cells is present in the bi-specific
antibody, however, the procedures available for the preparation
of that antibody, particularly when using hybridoma technology,
can lead to a complex mixture of antibodies, some of which may
show T-cell toxicity even though the bi-specific antibody does
15   not. The various types of different antibody which may be
present in the mixture obtained on preparing a bi-specific
antibody molecule from two hybridomas not expressing a myeloma
derived light chain are illustrated in the Figure 1 which
appears at the end of the specification. In theory, every
20   possible combination of the two different light chains and two
different heavy chains (shown in the Figure as black for those
having T-cell binding activity and white for those having
immunoglobulin binding activity) can occur and in practice some
antibodies of each type may be presumed to be obtained although
25   not necessarily in equal proportions. It is only in the case
where the bi-specific antibody molecule heavy chain having a
T-cell binding affinity will not mediate the killing of T-cells
by an Fc receptor mediated killing mechanism, such as ADCC or
complement activation, that none of the types of antibody will
30   be capable of destroying T-cells. In a case where this heavy
chain will cause the killing of T-cells via an Fc receptor
mediated killing mechanism, types 2 and 4 will be toxic via this
route irrespective of the nature of the immunoglobulin- binding
heavy chain, and types 1 and 5 will be toxic in addition to
35   types 2 and 4 when the immunoglobulin-binding heavy chain is not

such as to negate the activity of the other heavy chain.
Accordingly even though the bi-specific type 1 monoclonal
antibody molecules do not cause the killing of T-cells owing to
the selection of an appropriate combination of heavy chains for
05 the anti-T cell and anti-immunoglobulin halves of the molecule,
types 2 and 4 will always possess the undesirable ability to
kill T-cells.

It will be seen therefore that in the case of human therapy
using bi-specific antibodies comprising different rat IgG
10 combinations the order of value (for the anti T-cell/
anti-immunoglobulin combination) is IgG2b/IgG2b < IgG2b/IgG2a
(or 2c) < IgG2a (or 2c)/IgG2a (or 2c) or IgG2a (or 2c)/IgG2b
since the first is toxic to T-cells as the bi-specific molecule,
the second produces toxic molecules in admixture with the
15 bi-specific molecule but the third and fourth do not produce
molecules toxic to T-cells and the other types of molecule
present in admixture act only as a diluent to the bi-specific
molecules. Other rat IgG combinations of particular interest
for human therapy are IgG2b/IgG1, which should not be toxic to
20 T-cells as the bi-specific antibody but will provide toxic
type 2 and 4 molecules, and especially IgG1/IgG2b which should
not be toxic to T-cells as types 1, 2, 4 and 5. Alternatively,
the four possible different combinations of IgG1 with IgG2a or
IgG2c may be used, which would all be expected to behave
25 generally similarly to the IgG2a (or 2c)/IgG2a (or 2c)
combinations described above. As regards the mouse, the
combinations of similar isotypes IgG1/IgG1, IgG2a/IgG2a and
IgG2b/IgG2b are generally somewhat less preferred (particularly
IgG2a/IgG2a for the reasons given for rat IgG2b/rat IgG2b above)
30 than all of the possible dissimilar isotype combinations of
IgG1, IgG2a and IgG2b, which are of interest. Some of these
dissimilar isotype combinations will of course be of greater
interest than others on similar reasoning to that applied in the
rat case so that, for example, combinations in which the anti-T
35 cell binding is provided by mouse IgG2a will be expected

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disadvantageously to provide toxic type 2 and 4 molecules. The
whole range of rat IgG/mouse IgG and mouse IgG/rat IgG
combinations are also of some interest.

In a further aspect of the present invention, therefore, the
05   mixture of antibodies containing the bi-specific antibody of the
invention which is produced by a hybridoma system derived from
two fused hybridomas or two other fusion partners may be
fractionated to enhance the proportion of the bi-specific
antibody molecules therein and preferably substantially to
10   separate the bi-specific antibody molecules from or at least to
reduce the proportion of other species which are undesirable,
i.e. any types of molecule which act as a diluent to the type 1
molecules and particularly any species which are toxic to
T-cells by an Fc mediated receptor mechanism.

15   The techniques used for such purification are broadly
analogous to those described in UK Patent Application 2,144,147A
where the product it is desired to purify is one of type 4,
being a monovalent monoclonal antibody. Such procedures include
the use of affinity chromatography, ion exchange chromatogrpahy
20   and chromatofocussing, particularly using fast protein liquid
chromatography (FPLC - Pharmacia Trade Mark) and high pressure
liquid chromatography (HPLC) techniques.

Affinity chromatography can be exploited using either an
antigen-containing column (for example an immunoglobulin) which
25   will select for those species of molecule having the correct
combination of heavy and light chains for specificity or,
alternatively, an anti-isotype or protein A column can be used
to separate on the basis of isotype. Ion exchange
chromatography relies on the fact that at different pHs the
30   charge on a protein varies as different side chains ionize so
that the binding of protein to a charged column can be affected
by ionic strength. A powerful application of ion exchange
chomatography involves the separation of fractions on a first
column at a first pH followed by the use of a second column, at
35   a second pH, the columns usually being of opposite charge so

that cation exchange chromatography is followed by anion
exchange chromatography, or vice versa. Chromatofocussing
relies on the fact that at a particular selected pH, the protein
has no net charge and will not bind to a charged column so that
05   similar mixed proteins are separated on a basis of their pI.
FPLC and HPLC offer different advantages and may be used in
combination.

      As indicated above, by an appropriate selection of
anti-T-cell heavy chain which does not exhibit T-cell toxicity
10   or by the combination with a toxic anti-T-cell heavy chain of an
anti-immunoglobulin heavy chain of a different species or
isotype it is generally possible to achieve the desired aim of
the present invention to provide a bi-specific antibody molecule
which substantially avoids the killing of T-cells. By the use
15   of purification techniques as indicated above it is possible to
remove other components toxic to T-cells which would otherwise
cause a composition containing the bi-specific antibody
molecules to exhibit T-cell toxicity.

      It will be appreciated that the bi-specific antibody may
20   generally be used in the form of a fragment retaining the
binding affinities of the whole molecule. More importantly,
however, a second way of avoiding the destruction of T-cell by
the bispecific antibodies of the present invention is to use the
antibody in the form of a fragment of the whole antibody which
25   retains the binding affinities but not any T-cell killing
capacity, particularly a F(ab')$_2$ fragment. This approach
depends on the fact that the F(ab')$_2$ portion of the antibody
molecule is still capable of binding to T-cells through one of
its heavy chain/light chain combinations but the lack of the Fc
30   portion of the molecule means that the killing of T-cells by an
Fc mediated receptor mechanism cannot occur. A further
advantage of using F(ab')$_2$ portions is that, being smaller, they
are more rapidly cleared by the kidneys.

      The preparation of the F(ab')$_2$ portion of the antibody
35   molecule requires the use of an appropriate enzyme system to

effect cleavage at a suitable point in the heavy chains to
thereby remove the Fc region of the heavy chains
(or alternatively at least that of the anti-T cell heavy chain)
whilst retaining the remaining portion of the two heavy chains,
05   each linked to its light chain and also to the other heavy chain
via disulphide bridges. It will be appreciated that different
species and isotypes have different amino acid sequences in the
cleavage area of the immunoglobulin and that an appropriate
enzyme specific for the amino acid sequence in question must be
10   used. Furthermore, where the two heavy chains differ in species
or isotype not all cysteine groups in one chain will necessarily
be connected through a bridge with a cysteine group in the other
chain and the possibility of certain modes of cleavage yielding
Fab rather than $F(ab')_2$ fragments must be considered. It will
15   therefore be necessary to select an enzyme, for example pepsin,
papain, V8 protease, etc., and conditions of pH, temperature and
time for its use which are appropriate to the bi-specific
antibody system in question in order to produce a $F(ab')_2$
fragment (or other fragment lacking only one Fc region)
20   retaining the two specificities of the whole antibody (but not
its ability to kill T-cells). Having selected the appropriate
enzyme and conditions, as identified by the production of a
product having the characteristics indicated above, the
techniques for the production of the $F(ab')_2$ fragments of the
25   bi-specific antibodies according to the present invention are
broadly similar to those described in the literature for the
preparation of the $F(ab')_2$ fragments of mono-specific antibodies.

The present invention thus includes (a) a $F(ab')_2$ fragment
of a bi-specific antibody molecule consisting of two heavy
30   chains and two light chains and having a first binding affinity
for a T-cell receptor capable of activating killing and a second
binding affinity for immunoglobulin, which immunoglobulin
binding affinity is not one which causes the bi-specific
antibody molecule to be directly targeted to cells present in
35   the species in which the T-cell receptor occurs, and also (b) a
process for the preparation of such a fragment which comprises
treating the antibody molecule with a suitable enzyme system to
effect cleavage thereof to yield this fragment.

It will be appreciated that the fractionation of the different antibody molecules of types 1 to 10 as discussed hereinbefore can still be of value where the F(ab')$_2$ fragment is used since, although the removal of other types toxic to T-cells

05   is not necessary in this instance, all of the other types 2 to 10 dilute the proportion of the single active type 1 in the preparation, whilst types 2, 4 and 5 will compete for binding to T-cells and types 3, 6 and 7 for binding to immunoglobulin. The other types, particularly types 2 to 7, may therefore

10   advantageously be reduced in proportion to type 1 or substantially removed. This fractionation may be carried out either before, or preferably after, formation of the F(ab')$_2$ fragment.

It will be appreciated that there has never been any

15   suggestion that the bi-specific antibodies and fragments thereof which are the subject of the present invention might be used in a medical context. The present invention therefore further includes (a) a bi-specific antibody molecule consisting of two heavy chains and two light chains and having a first binding

20   affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, which immunoglobulin binding affinity is not one which causes the bi-specific antibody molecule to be directly targeted to cells present in the species in which the T-cell receptor occurs, or a

25   fragment thereof retaining the binding affinities of the whole molecule, for use in surgery, therapy or diagnosis and also (b) the use of a bi-specific antibody molecule consisting of two heavy chains and two light chains and having a first binding affinity for a T-cell receptor capable of activating killing and

30   a second binding affinity for immunoglobulin, which immunoglobulin binding affinity is not one which causes the bi-specific antibody molecule to be directly targeted to cells present in the species in which the T-cell receptor occurs, or a fragment thereof retaining the binding affinities of the whole

35   molecule, for the manufacture of a medicament for use in the treatment of neoplastic or other disease.

The bi-specific antibodies and fragments thereof described herein may be formulated for use in various ways, which will

however, usually involve the use of a physiologically acceptable
diluent or carrier which will conveniently be sterile and
preferably also pyrogen-free for certain uses. This may take
various forms, for example phosphate buffered saline, saline,
05   balanced salt solution and dextrose solution. However,
phosphate buffered saline may be mentioned especially as often
being suitable.

The composition may, if desired, be presented in unit dosage
form, i.e. in the form of discrete portions containing a unit
10   . dose, or a multiple or sub-unit dose.

The anti-target cell antibodies described herein may also be
utilised in fragment, for example F(ab')$_2$, form although the
advantages in doing this are less than those described above for
the bi-specific antibodies. Moreover, the anti-target cell
15   antibodies, or fragments thereof, may conveniently be formulated
in a broadly similar manner to the bi-specific antibody
molecule, or fragment thereof. Indeed, it may be appropriate
either to incorporate the two types of antibody into the same
composition or into a kit, although the former may result in
20   conjugate formation as described hereinafter. The present
invention thus includes (a) a composition comprising a
bi-specific antibody molecule consisting of two heavy chains and
two light chains and having a first binding affinity for a
T-cell receptor capable of activating killing and a second
25   binding affinity for immunoglobulin, which immunoglobulin
binding affinity is not one which causes the bi-specific
antibody molecule to be directly targeted to cells present in
the species in which the T-cell receptor occurs, or a fragment
thereof retaining the binding affinities of the whole molecule,
30   and an antibody molecule having a binding affinity for target
cells, or a fragment thereof having such an affinity, and also
(b) a kit comprising in association a bi-specific antibody
molecule consisting of two heavy chains and two light chains and
having a first binding affinity for a T-cell receptor capable of
35   activating killing and a second binding affinity for
immunoglobulin, which immunoglobulin binding affinity is not one
which causes the bi-specific antibody molecule to be directly
targeted to cells present in the species in which the T-cell

receptor occurs, or a fragment thereof retaining the binding affinities of the whole molecule, and an antibody molecule having a binding affinity for target cells, or a fragment thereof having such an affinity.

05   Both types of antibody or fragment thereof may be administered in various ways, for example intravenously, intraperitoneally or possibly intracerebrally, the mode of administration being selected to be appropriate to the type and localisation of the tumour or other target cells and also for

10 ease of administration by the clinician and for the safety of the patient. In general, however, parenteral administration, and particularly intravenous injection, will often be used. The two types of antibody may be employed simultaneously, separately or sequentially but it will usually be the case that the

15   anti-target cell antibody will be administered either before or together with the bi-specific antibody and where the latter is the case, a suitable time delay will often be from 0.5 to 24 hours. As regards dosages of the two types of antibody, or fragment thereof, the exact dosages will depend upon the

20   potency of the reagents, the tumour or other disease burden of the patient and the patient's body weight/surface area ratio. It may, however, be stated by way of guidance that a dosage of between 1 to 25 mg of each antibody or fragment, for example approximately the same amount of each, will often be suitable,

25   conveniently used in a 7 to 10 day regimen involving 1 dose of each per day, i.e. a 10 day course of treatment involving the administration of a total dosage of 10 to 250 mg of each antibody or fragment to the patient. It will be appreciated however that dosages outside this range may be used where

30   appropriate although a particular advantage of the present invention is the low dosages which may be used in many cases, i.e. towards the lower end of the range stated or even below it, thereby possibly even avoiding the setting up of an immune response to the bi-specific antibody molecules and thus allowing

35   repeated usage.

It will be appreciated that the present invention includes a method for the treatment of a neoplastic, viral or parasitic disease which comprises administering to a patient a bi-specific

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antibody molecule consisting of two heavy chains and two light
chains and having a first binding affinity for a T-cell receptor
capable of activating killing and a second binding affinity for
immunoglobulin, which immunoglobulin binding affinity is not one
05 which causes the bi-specific antibody molecule to be directly
targeted to cells present in the species in which the T-cell
receptor occurs, or a fragment thereof retaining the binding
affinities of the whole molecule, and an antibody molecule.

As an alternative to the _in vivo_ use described hereinbefore,
10 the bi-specific antibodies may be used in conjunction with the
anti-tumour antibodies for the removal of neoplastic cells from
bone marrow _in vitro_, thereby allowing autologous bone marrow
transplantation to be used in the treatment of malignancy.

The use of the bi-specific antibody molecules of the present
15 invention in indirect ECR offers many benefits over direct ECR
as envisaged by Staerz and Bevan. Possible variations upon the
simple use of the bi-specific antibody molecules together with
the anti-target cell antibodies _in vivo_ or _in vitro_ as described
hereinbefore include the removal of some peripheral blood
20 mononuclear cells from the patient and the treatment of these
cells briefly with anti-T-cell antibodies, such as the
monoclonal antibodies YTH 12.5.14 or 12.5.14.2 described in
Example 2, in order to activate the cells. A panel of
anti-tumour monoclonal antibodies is then selected, for example
25 against differentiation antigens, surface immunoglobulins,
tumour associated antigens, etc., and appropriate antibodies or
combinations of antibodies are used to target the tumour cells
for lysis by activated effectors which are reintroduced into the
body several hours later in the presence of the bi-specific
30 antibody molecules, allowing excess anti-tumour cell antibodies
to be eliminated. In addition to versatility, an important
advantage of indirect ECR versus direct ECR is that both
preactivated cytotoxic 1-lymphocyte (CTL) and K-cell populations
may be recruited to destroy a tumour target. If the Fc portion
35 of a bi-specific antibody molecule with anti-T-cell and
anti-target cell affinities is able to bind CD16 on activated
K-cells or Fc receptors on other effector cell populations such
as monocytes and neutrophils, and to bind CD3 on CTL, then the

antibody will not be ideal for therapeutic use as it may induce
reciprocal killing between CTLs and K-cells or monocytes, etc.,
similar to that observed between CTLs in the presence of
bivalent CD3 Mab. Therefore the efficient use of direct ECR

05 requires that the FcγRlow on K-cells or Fc receptors on effector
cells must be either blocked, for example with a suitable CD16
monoclonal antibody, or the heavy chains of the bi-specific
antibody molecule must be selected so that the Fc portion does
not bind to FcγRlow or other Fc receptors. Alternatively, the

10 Fc portion of an anti-T-cell/anti-target cell bi-specific
antibody molecule may be rendered inactive by producing $F(ab')_2$
fragments. Unfortunately, these processes eliminate the
potential for K-cell mediated ADCC against a target cell coated
with the bi-specific antibody. It is advantageous to utilise

15 both ECR and ADCC to focus killing against a target cell and
K-cell killing can be exploited using indirect ECR because the
anti-target cell antibodies can be administered separately from
the bi-specific antibody molecules. In such a context it is
preferred to select anti-tumour cell or other anti-target cell

20 antibodies of the rat IgG2b isotype which have been shown to
mediate potent K-cell killing by ADCC and the technology now
exists to class-switch other isotype of rat antibodies to the
IgG2b isotype. On infusing treated effector cells back into the
patient, the activated K-cells home to the tumour site and kill

25 antibody-coated target cells via ADCC. Additionally,
bi-specific antibody molecules selected for having a
non-functional Fc portion mediate indirect ECR by focussing the
CTL response against the same population of target cells.

It will be appreciated that the bi-specific antibody

30 molecules according to the present invention potentially allow
the administration of different antibody treatments at various
time intervals. This permits clearance of an excess of a first
anti-target cell antibody so that a second anti-target cell
antibody can home efficiently via its antiglobulin specificity

35 to the tumour cells bound by the remaining first antibody. This
will reduce the formation of aggregates which can be removed
very efficiently by cells of the reticuloendothelial system,
etc. Additionally, with indirect ECR the use directly of an

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appropriate anti-target cell antibody will allow the activated
K-cells to mediate ADCC against the target cell. This is
followed by the administration of bi-specific antibody molecules
when the CTL population is also recruited to kill the same
05   target cell via indirect ECR.

In addition to their use as described hereinbefore the
bi-specific antibody molecules consisting of two heavy chains
and two light chains and having a first binding affinity for a
T-cell receptor capable of activating killing and a second
10   binding affinity for immunoglobulin may be used, either as the
whole molecule or as a fragment thereof retaining the binding
affinities of the whole molecule, in the preparation of
bi-specific antibody conjugates of use in direct ECR having a
first binding affinity for a T-cell receptor capable of
15   activating killing and a second binding affinity for target
cells. In this aspect of the present invention the bi-specific
antibody molecules provide an intermediate capable of use to
prepare a range of bi-specific antibody conjugates having the
same T-cell binding affinity but differing tumour cell or other
20   target cell binding affinities. It will be appreciated that
such a conjugate does not have a free immunoglobulin binding
affinity and that this affinity of the bi-specific antibody
molecule component of the conjugate does not therefore have to
be limited in the same way as the bi-specific antibody molecules
25   for indirect ECR use described previously, although in practice
similar preferences as to the nature of the immunoglobulin
binding affinity are likely to apply.

The present invention therefore includes a bi-specific
antibody conjugate having a first binding affinity for a T-cell
30   receptor capable of activating killing and a second binding
affinity for target cells, characterised in that the conjugate
comprises a bi-specific antibody molecule having a first binding
affinity for a T-cell receptor capable of activating killing and
a second binding affinity for immunoglobulin linked to an
35   antibody molecule having a binding affinity for target cells, or
a fragment thereof retaining the binding affinities of the whole
conjugate.

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For this alternative use, the bi-specific antibody molecules
of the present invention are combined in vitro with an antibody
having a binding affinity for target cells such as is described
herein, the conjugate then being administered in vivo. Although
05   the conjugates could be prepared by chemical linkage it is of
course preferable to use the anti-immunoglobulin affinity of the
bi-specific antibody molecule for the anti-target cell antibody
to provide the linkage. The conjugates may therefore
conveniently be prepared by the simple admixture of solutions of
10   a bi-specific antibody molecule having a first binding affinity
for a T-cell receptor capable of activating killing and a second
binding affinity for immunoglobulin and an antibody having a
binding affinity for target cells. It is preferred that the
bi-specific antibody conjugates so prepared are not isolated but
15   are administered directly to the patient. Conveniently,
therefore, the preparation may be effected in a suitable medium
for this purpose so that, for example, the two components may be
contained in equal volumes of sterile physiological saline. The
mixture may then be left for a suitable period for reaction to
20   be complete but not to allow any substantial degree of
aggregation, such as for up to 1 hour, for example 30 minutes,
and may then be administered, particularly parenterally.

All of the preferences expressed hereinbefore in relation to
the separate bi-specific antibody molecules and anti-target cell
25   antibodies and their use will of course continue to apply when
these are components of the bi-specific antibody conjugates.
Thus, for example, a $F(ab')_2$ or other fragment of the
bi-specific antibody molecule may be combined with the
anti-target cell antibody or a $F(ab')_2$ or other fragment
30   thereof. Formulation for therapeutic use will be effected
similarly and doses will be similar bearing in mind that the
conjugates are a combination of the two separate components so
that the dosages indicated for these separate component will be
combined to provide the appropriate dosages for the conjugates.
35   The present invention is illustrated by the following
Examples.

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Example 1

Preparation of h,bridoma producing monoclonal antibodies
specific for the human CD3 antigen and for rat immunoglobulin

(1) Anti-CD3 component

05   The Lou rat myeloma cell line Y3-Ag 1.2.3 (CNCM, I-078) is
fused with spleen cells from a DA rat immunised with human
lymphocytes according to the procedure described by Clark and
Waldmann, Methods in Hematology, 1986, 13, 1-20, and the fusion
mixture worked up as described therein selecting for hybridomas

10   producing monoclonal antibodies having specificity for the human
CD3 antigen defined by reactivity with all human peripheral
T-cells, cross inhibition with the mouse monoclonal antibodies
such as UCHT-1 (Burnset et al. Journal of Immunology, 1982, 129,
1451) and OKT-3 (US Patent 4361549), and immunoprecipitation.

15   The hybridoma is hypoxanthine-guanine phosphoribosyl transferase
positive (HPRT$^+$) and expresses one spleen cell-derived light
chain and a second myeloma-derived light chain of the kappa-1a
allotype.

        Selection is made for myeloma light chain loss variants by

20   cell cloning on semi-solid agar (Clark and Waldmann, ibid) and
then assaying for the loss of rat kappa-1a allotype expression
using a sensitive red cell haemagglutination assay (Clark,
Methods in Enzymology, 1986, 121, 548-556). The variant
selected is cloned on semi-solid agar and then maintained in

25   culture in Iscoves modification of Dulbecco's medium (IMDM –
Gibco Europe) supplemented with 1 to 5% v/v foetal calf serum
(FCS) and buffered with bicarbonate using 5% CO_2 in air.

        For short term handling of the cells the bicarbonate is
replaced by extra hepes buffer and NaCl to maintain the ionic

30   strength.

(2) Anti-rat immunoglobulin component

        The BALB/C myeloma cell line NSI/Ag 4.1 is fused with spleen
cells from a SJL mouse strain immunised with pooled rat IgG in
complete Freund's adjuvant (Springer et al, Hybridoma, 1982, 1,

35   257-273).

The selected hybridoma produces a monoclonal antibody having specificity for a particular rat allotype but this is not one corresponding to the immunoglobulin produced by the hybridoma (1), thereby avoiding auto-reactivity. Selection is

05 made for a variant of this hybridoma which is HPRT negative by effecting culture in increasing concentrations of a medium containing the selective drug 8-azaguanine (Clark and Waldmann, ibid). The selected HPRT⁻ variant is cloned and cultured as for the hybridoma (1).*

10 (3) Hybridoma-hybridoma cell fusion

Prior to fusion the hybridoma (1) is treated in phosphate buffered saline (PBS) with 10 mM iodoacetamide for 30 minutes at 0°C. The HPRT⁺ iodoacetamide-poisoned cells are washed with HEPES buffered IMDM to remove excess inhibitor and then mixed

15 with the HPRT⁻ hybridoma (2) cells in three different proportions:- (a) 3.5×10^7 cells hybridoma (1) + 3.5×10^6 cells hybridoma (2) (10:1); (b) 3.5×10^7 cells hybridoma (1) + 3.5×10^7 cells hybridoma (2) (1:1); and (c) 3.5×10^7 cells hybridoma (1) + 3.5×10^8 cells hybridoma (2). In each case the

20 cells are mixed in HEPES buffered IMDM and pelleted at 200 x g. Cell fusion is induced by treating the cell pellet for 2 minutes with 1 ml of a 50% w/v solution of polyethylene glycol 1500 in PBS whilst stirring (Clark and Waldmann, ibid). The cells are then washed once with HEPES buffered IMDM, resuspended in

25 bicarbonate buffered IMDM containing 5% v/v foetal calf serum, plated out into 24 x 2 ml culture well plates at four concentrations from 8×10^5 to 2×10^5 and cultured at 37°C under 5% CO_2. On the following day a control containing iodoacetamide-treated but infused cells is typically totally

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* In a first variant of this procedure the hybridoma (1) is 8-azaguanine resistant and the hybridoma (2) is poisoned with iodoacetamide in step (3) and in a second variant 6-thioguanine is used instead of 8-azaguanine in either the original or first variant procedures, and in a third variant the hybridoma (2) is a myeloma light chain loss variant as well as or instead of the hybridoma (1), or one or both hybridomas are derived from a myeloma which does not express a light chain.

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non-viable, whilst in the cultures of the fused cells the
majority of cells are observed to be viable.

After culture for 24 hours, selection is commenced with
hypoxanthine, aminopterine and thymidine (HAT selective medium -
05   Clark and Waldmann, ibid). the HPRT⁻ hybridoma (2) cells being
non-viable in this medium. Culture in the HAT selective medium
is continued for 2 or more weeks and the following assays are
then employed to screen for hybrid cell lines producing
bi-specific antibody. Functional human CO3 antibody is detected
10 . using immunofluorescence to demonstrate binding on HP8-ALL
(Adrian and Hutton, Journal of Clinical Investigation, 1983, 71,
1649-1660), a human T-cell leukaemia cell line, such binding
indicating the presence of both the heavy and the light chain of
the hybridoma (1). Functional anti-rat immunoglobulin activity
15   is detected using a rat antibody with anti-mouse Thy-1 antigen
or other irrelevant specificity (which is of the appropriate
allotype in relation to hybridoma (2)) fixed to the microtitre
wells. Antibody capable of binding to such an antibody is
detected with biotinylated YA 9/36.39 (Clark, Ph.D. thesis,
20   University of Cambridge, 1982), a reagent specific for mouse
IgG2, The plate binding assay is again employed to screen for
antibodies containing mixed immunoglobulin chains derived from
both parental cells, the biotinylated YA 9/36.69 being replaced
by a biotinylated monoclonal antibody specific for the rat IgG
25   sub-class of the hybridoma (1). Cells from wells which are
strongly positive in all three assay procedures are cloned twice
in semi-solid agar and a suitable clone is selected, this being
maintained in culture as for the mono-specific hybridoma (1).*

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* In a variant of this procedure the anti-rat Ig hybridoma (2)
is replaced by a hybridoma specific for Ig of another species,
for example the mouse or the human. In a further variant, which
may conveniently be combined with the first, one or both of the
hybridomas (1) and (2) are replaced by a hybridoma derived from
a human myeloma, for example as described in European Patent
Applications 0052403 and 0142544, in UK Patent 2035873 and in
US Patent 4529694.

Example 2

Preparation of rat IgG2b anti-human CO3/mouse IaG2a anti-rat
immunoglobulin bi-specific hybridoma LHC 49.18.2

      The procedure of Example 1(3) was followed using as
05   hybridoma (1) the hybridoma YTH 12.5.14 (this is identical to
the sister clone YTH 12.5.22 described by Cobbold and Waidmann,
Nature, 1984, 308, 460-462) and as hybridoma (2) the hybridoma
RG 11/15.5 (Springer et al, ibid). YTH 12.5.14 produces a rat
IgG2b monoclonal antibody and expresses a myeloma-derived kappa
-10   light chain of the la-allotype. The myeloma chain loss variant
YTH 12.5.14.2 secretes a single light chain unreactive with all
tested anti-rat kappa reagents and which may therefore be
presumed to express a light chain of the lambda class.
RG 11/15.5 produces a mouse IgG2a monoclonal antibody specific
15   for the rat kappa 1b allotype light chain and expresses a kappa
light chain.

      The fusion mixture was screened using biotinylated
NORIG 7.16.2 (Hale et al, Journal of Immunology, 1985, 134,
3056), a reagent specific for rat IgG2b in the third assay
20   procedure. The bispecific anti-human CO3/anti-rat kappa 1b
allotype light chain hybridoma LHC 49.18.2 was selected
following the cloning procedure.

Example 3

Preparation of mouse IgG2a anti-human CO3/mouse IgG2a anti-rat
25   immunoglobulin bi-specific hybridoma LHO 6.23

      The procedure of Example 1 was followed again using as
hybridoma (2) the hybridoma RG 11/15.5 referred to in Example 2
and in this case using as hybridoma (1) the hybridoma OKT3.5.2
which is a reclone of the mouse IgG2a anti-human CO3 hybridoma
30   OKT3 referred to in US Patent 4361549 and deposited with the
ATCC, Rockville, USA, under the accession number CRL 8001.
OKT3.5.2 has identical properties to OKT3, which is derived from
the mouse myeloma P3-X63-Ag8U1, that secretes a mouse kappa
light chain, through a fusion with spleen cells from a CAF1
35   mouse immunised with E rosette positive-purified human T-cells.

The fusion mixture was screened with an immunoglobulin
isotype assay using a suitable rat IgG antibody with a kappa 1b
allotype light chain fixed to the microtitre wells and
biotinylated YA9/36.69 to detect RG11/15.5 specificity and

05   complement mediated lysis of human T-cells to detect OKT3
specificity by indirect fluorescence. The bi-specific
anti-human CD3/anti-rat kappa 1b allotype light chain hybridoma
LHD 6.23 was selected following the cloning procedure.

Example 4

10   Preparation of mouse IgG2a anti-human CD3/mouse IgG2a anti-rat
immunoglobulin bi-specific hybridoma LH8 63.10
The procedure of Example 1 was followed using the hybridoma
OKT3.5.2, which is described in Example 3, as the hybridoma (1)
and as hybridoma (2) the hybridoma NORIG 7.16.2 which is an HL

15   secreting hybridoma derived by a fusion between spleen cells
from a BALB/c mouse immunized with rat anti-pertussis/pertussis
immune complexes and the non-producing mouse myeloma NSO/u
(NORIG 7.16.2 is a subclone of identical properties to the
NORIG 7.16 described by Hale et al, ibid). NORIG 7.16.2

20   produces an anti-rat IgG2b mouse IgG2a monoclonal antibody
expressing a kappa light chain. The 8-azaguanine resistant HL
clone used was NORIG 7.16.2 AG. Following fusion, the screening
procedures involved complement mediated lysis of human T-cells
to detect OKT3 specificity by indirect fluorescence and an ELISA

25   assay using plates coated with an irrelevant rat IgG2b
antibody. NORIG 7.1.62 specificity was detected using a
specific biotinylated anti-mouse Ig reagent followed by
streptavidin-peroxidase (both from Amersham plc, England).
Following the cloning procedure the bi-specific anti-human

30   CD3/anti-rat IgG2b hybridoma LH8 63.10 was selected.

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Example 5
Production of monoclonal antibodies from bi-specific hybridoma
     The bi-specific hybridoma produced in Example 1, 2, 3 or 4
is maintained in low serum culture (IMDM containing 1% v/v FCS)
05   for 48 hours at 37°C using 5% CO_2 in air. The culture
supernatant is then concentrated by precipitation with ammonium
sulphate added to a level of 50% w/v. The precipitate is
redissolved in the minimum volume of water and is desalted
into 20 mM tris buffer at pH 8.0 on Sephadex G25. The antibody
10   is fractionated by hplc ion exchange chromatography on a TSK-5PW
column (LKB Ltd.). Pooled fractions are desalted into PBS for
assay.

Example 6
Tests of activity of rat IgG2b/mouse IgG2a bi-specific
15   monoclonal antibodies in effector cell retargeting
(A)
(1) Preparation of human cytotoxic effector cells
     Venous blood was collected from healthy donors and was
defibrinated using glass beads. Mononuclear cells were isolated
20   from the interface following density gradient centrifugation on
Ficoll-Hypaque and were then washed into bicarbonate buffered
IMDM containing 5% v/v FCS. These cells were divided into
tissue culture bottles at 1 x 10^6 cells per ml in the same
culture medium, the bottles having been pre-coated with
25   anti-human CD3 antibodies, in particular those produced by the
hybridoma YTH 12.5.14.2 of Example 2. After 3 days in culture
at 37°C the effectors were subjected to a 7 day expansion in the
same medium containing 10 U/ml rIL2 (Cetus).
     The blast cells produced in this way also contain 10-15% Fc
30   receptor positive cells (as detected by Fc rosetting) which are
able to mediate ADCC. These ADCC effectors were inhibited by
pre-incubating the once-washed effector cells in the same medium

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with a 1/600 dilution of the anti-Fc receptor antibody CLB-Fcr
gran I (Tetteroo et al, Leucocyte Typing II, 1985, Volume 3,
page 27 - Springer Verlanger, New York) for 15 minutes at room
temperature. This anti-CO16 antibody blocks K-cell mediated
05   ADCC.

(2) Effector cell retargeting assay

     The mouse Thy-1 positive cell line BW 5147 (CRL 1588,
PHLS European Collection of Animal Cell Cultures, Porton Down,
England) was used as the target cell line. Cells were
10   maintained in exponential phase in IMDM containing 2% v/v FCS
until required for assay. Approximately 5 x 10^6 cells were then
spun down at 200 x g and the pellet resuspended in 150 µl IMDM
containing 300 µCi ^{51}Cr-sodium chromate. The cells were
incubated at 37°C for 1 hour and were washed into HEPES buffered
15   IMDM. The target cells were then washed and divided into two
tubes. The cells in the first tube were resuspended in 500 µl
of HEPES buffered IMDM medium for analysis of direct ECR and the
cells in the second tube were resuspended in a similar volume of
the same medium containing approximately 100 µg/ml of anti-Thy-1
20   monoclonal antibodies derived from the hybridoma YBM 29.2.1 (see
Cobbold et al, ibid) for analysis of indirect ECR. After a 1
hour incubation at room temperature the cells in the second tube
were washed once to remove excess YBM 29.2.1 and were
resuspended in the same medium at a level of 2 x 10^5 viable
25   cells/ml.

     Two-fold dilutions of effector cells were set up in 50 µl
volumes in U-bottom microtitrre wells and equal vol8 hybridoma,
(abelled Thy-1 coated or uncoated target cells were added in
a 50 µl volume of HEPES buffered IMDM medium to provide an
30   effector:target cell ratio ranging from 32:1 to 0.5:1. Suitable
dilutions of the antibodies to be tested for effector cell
retargeting were added in a 100 µl of HEPES buffered IMDM to the
wells and incubation was effected at 37°C for 4 hours, after
which 100 µl of medium per well was collected for a
35   determination of the released radioactivity by measurement of
gamma radiation using a Phillips gamma counter, model PW 4800.

(3) Determination of activity of LHC 49.18 antibodies and
SHN 20.12 antibodies

   Supernatants were used (the culture being obtained as in
Example 5) of (a) a culture of Y3-Ag 1.2.3 myeloma cells as a

05 control, (b) a 1:1 v/v mixture from cultures of the parental
hybridomas YTH 12.5.14.2 and RG 11/15.5 of the LHC 49.18
hybridoma, (c) a culture of LHC 49.18 hybridoma cells, and (d) a
culture of SHN 20.12 hybridoma cells [SHN 20.12 is a rat IgG2b
anti-human CD3/rat IgG2c anti-mouse Thy-1 bi-specific hybridoma

10 prepared as described in Example 3 of Australian Patent
Application 87/81569] and (e) a 1:1 v/v mixture from cultures of
the parental hybridomas YBM 29.2.1 and YTH 12.5.14.2 of the
SHN 20.12 hybridoma.

   In a first set of experiments the assay procedure described

15 under (2) above was used but with a constant effector:target
cell ratio of 15:1 and with a dilution of supernatant (in 100 μl
volume) ranging from 1/100 to 1/12800. The control Y3-Ag 1.2.3
cells and the parental mixture showed no significant activity as
measured by the level of ^{51}Cr release with both unlabelled and

20 labelled target cells (but see below in relation to parental
mixture). As also expected, the SHN 20.12 antibodies showed a
marked level of ^{51}Cr release for both types of target cell
whilst the LHC 49.18 antibodies, which unlike the SHN 20.12
antibodies will cause only indirect ECR, gave a marked level

25 of ^{51}Cr release with the labelled cells but only a similar level
of ^{51}Cr release to the control and parental mixture for the
unlabelled cells. In a second set of experiments the
effector:target cell ratio was varied from 32:1 to 0.5:1 as
described under (2) above in conjunction with supernatants at a

30 single dilution of 1/100 in 100 μl volume. The results obtained
are shown in Figures 2a and 2b which relate to unlabelled and
labelled target cells, respectively. The SHN 20.12 antibodies,
which effect direct ECR, were found to be active with both
uncoated and coated target cells (plot shown only in Figure 2a

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for clarity of other plots in Figure 2b) whilst the LHC 49.18
antibodies, which effect indirect ECR, are as expected effective
only with coated target cells (plot shown only in Figure 2b for
clarity of other plots in Figure 2a). However, the killing
05   produced by indirect ECR with the LHC 49.18 antibodies as shown
in Figure 2b will be seen to be at least as effective as the
killing produced by direct ECR with the SHN 20.12 antibodies as
shown in Figure 2a. (The slight activity observed for the
parental mixtures is believed to be attributable to a small
10   number of spontaneously arising microaggregates.)
(B)

The experiments described in (A) above were repeated using
both the mouse lymphoma BW 5147 as a target and also the mouse
Thy-1 positive lymphoma cell line EL-4 (A.T.C.C. reference
15   number TIB 40) which was prepared for use in the same way as
described in (A) for the BW 5147 cells. Each of the BW 5147 and
EL-4 target cells were used in both unlabelled and labelled
forms as descirbed in (A) using for labelling monoclonal
antibodies from the anti-Thy-1 rat IgG2c, klb hybridoma YBM 29.2
20   as in (A) or alternatively, monoclonal antibodies from the
anti-Thy-1 rat IgG2b, klb hybridoma YTH 154.7.7 (Cobbold et al,
Molecular Biology and Medicine, 1983, 1, 285-304) or the
anti-LC/7200 rat IgG2a, klb hybridoma YBM 42.2 (Stenning et al,
Molecular Biology and Medicine, 1983, 1, 95-115). The labelled
25   target cells were added to microtitre plates containing two-fold
dilutions of effectors and a 1/100 dilution of supernatant was
added (the cultures being obtained as in Example 5) of one of
(a) a culture of Y3-Ag 1.2.3 myeloma cells as a control,
(b) a 1:1 v/v mixture of cultures of the parental hybridomas,
30   YTH 12.5.14.2 and RG11/15.5, of the LHC 49.18 hybridoma, and
(c) a culture of the hybridoma LHC 49.18.

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Figures 3a and 3b show the results obtained with BW 5147
cells coated with YBM 29.2.1 and YBM 42.2 antibodies,
respectively. Figures 3c and 3d show the results obtained with
EL-4 cells coated with YBM 29.2.1 and YBM 42.2 antibodies,

05   respectively. The results obtained with both types of target
cells coated with YTH 154.7 antibodies were similar to those
obtained with YBM 29.2.1-coated target cells and are therefore
not illustrated. Unlabelled target cells of both types were, as
expected, not killed in the presence of any of the three types

10   of supernatant.

It will be seen from Figure 3 that the LHC 49.18 antibodies,
but not the control or the mixture of parental antibodies,
achieved a good level of killing of both BW 5147 and EL-4 via
anti-target-cell antibodies binding to the two antigens Thy-1

15   and leucocyte common antigen.

CLAIMS

1. A bi-specific antibody molecule consisting of two heavy chains and two light chains and having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, which immunoglobulin

05 binding affinity is not one which causes the bi-specific antibody molecule to be directly targeted to cells present in the species in which the T-cell receptor occurs, or a fragment thereof retaining the binding affinities of the whole molecule.

2. An bi-specific antibody molecule consisting of two heavy

10 chains and two light chains and having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, which immunoglobulin binding affinity is for immunoglobulin other than endogenously synthesised and expressed cell surface immunoglobulins of the

15 species in which the T-cell receptor occurs.

3. An antibody molecule or fragment thereof according to Claim 1 or 2, in which the receptor is a human T-cell receptor.

4. A bi-specific antibody molecule consisting of two heavy chains and two light chains and having a first binding affinity

20 for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, which immunoglobulin binding affinity is for immunoglobulin of a different species than that in which the T-cell receptor occurs.

5. An antibody molecule or a fragment thereof according to

25 Claim 4, in which the receptor is a human T-cell receptor.

6. An antibody molecule or fragment thereof according to Claim 5, in which the immunoglobulin binding affinity is for rat and/or mouse immunoglobulin.

7. An antibody molecule or fragment thereof according to any of

30 the preceding claims, in which the second binding affinity is for immunoglobulin which does not correspond to the immunoglobulin of either light chain or either heavy chain in the molecule.

8. An antibody molecule or fragment thereof according to any of Claims 1 to 7, in which the two heavy chains each separately are derived from the human, rat or mouse and are of the IgG class of immunoglobulins.

05   9. An antibody molecule or fragment thereof according to any of Claims 1 to 8, in which the two heavy chains in the molecule are selected to mitigate the killing of human T-cells by the molecule.

10. An antibody molecule or fragment thereof according to
10   Claim 9, in which T-cell killing is mitigated by the use of a first heavy chain having a T-cell binding affinity which is substantially ineffective at promoting killing through Fc receptor mediated killing mechanisms.

11. An antibody molecule or fragment thereof according to
15   Claim 10, in which said heavy chain is derived from the rat and is of the IgG2a, IgG2c or IgG1 sub-class.

12. An antibody molecule or fragment thereof according to Claim 11, in which the two heavy chains are of the isotypes, for the anti-T-cell/anti-immunoglobulin cell specificities
20   respectively, of IgG2a/IgG2a, IgG2a/IgG2c, IgG2c/IgG2a, IgG2c/IgG2c, IgG2a/IgG2b or IgG2c/IgG2b.

13. An antibody molecule or fragment thereof according to Claim 10, in which said heavy chain is derived from the mouse and is of the IgG2b, IgG1 or IgG3 subclass.

25   14. An antibody molecule or fragment thereof according to Claim 9, in which the first heavy chain having a T-cell binding affinity is effective at promoting killing through Fc receptor mediated killing mechanisms but T-cell killing is mitigated by the presence of a second heavy chain having immunoglobulin
30   binding affinity which renders said first heavy chain less effective.

15. An antibody molecule or fragment thereof according to Claim 14, in which the first and second heavy chains are of a different species, isotype or allotype.

16. An antibody molecule or fragment thereof according to Claim 14, in which the first heavy chain is rat IgG2b or mouse IgG2a.

17. An antibody molecule according to Claim 15 or 16, in which the first and second heavy chains are of the same class.

18. An antibody molecule or fragment thereof according to Claim 17, in which the heavy chains are derived from the rat and are of the isotypes, for the first chain/second chain respectively, IgG2b/IgG2a or IgG2b/IgG2c or are derived from the mouse and are of the isotypes, for the first chain/second chain respectively, IgG2a/IgG1 or IgG2a/IgG2b.

19. A bi-specific antibody molecule consisting of two heavy chains and two light chains and having a first binding affinity for a human T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, which immunoglobulin binding affinity is for non-human immunoglobulin, characterised in that the two heavy chains in the molecule are of a different species or isotype, or a fragment thereof retaining the binding affinities of the whole molecule.

20. An antibody molecule or fragment thereof according to Claim 19, in which the two heavy chains are derived from different species among the human, mouse and rat.

21. An antibody molecule or fragment thereof according to Claim 19, in which the two heavy chains are either derived one from the rat and the other from the mouse or are of different rat or of different mouse isotypes.

22. An antibody molecule according to any of Claims 19 to 21, in which the first and second heavy chains are of the same class.

23. An antibody molecule or fragment thereof according to Claim 22, in which the heavy chains are both derived from the rat and are of the isotypes, for the anti-T-cell/anti-immuno-globulin cell specificities respectively, IgG2a/IgG2c, IgG2c/IgG2a, IgG2a/IgG2b or IgG2c/IgG2b or are both derived from the mouse and are of the isotypes, for the anti-T-cell/anti-immunoglobulin cell specificities respectively, of IgG1/IgG2b, IgG2b/IgG1, IgG1/IgG2a or IgG2b/Ig2a.

24. An antibody molecule or fragment thereof according to any
of Claims 14 to 18, which is substantially free from antibody
molecules which have two of said first heavy chains and in which
at least one of these heavy chains is in combination with its
05 corresponding light chain, and from fragments of said antibody
molecules retaining the binding affinities of the whole molecule.
25. An antibody molecule or fragment thereof according to any
of Claims 19 to 23, which is substantially free from antibody
molecules which have two heavy chains with said first binding
10 affinity and in which at least one of these heavy chains is in
combination with its corresponding light chain, and from
fragments of said antibody molecules retaining the binding of
the whole molecule.
26. A fragment of an antibody molecule according to any of the
15 preceding claims which is either the F(ab')$_2$ fragment of the
whole molecule or a fragment of the whole molecule lacking only
that Fc region which is that of the heavy chain having a T-cell
binding affinity.
27. A fragment of a bi-specific antibody molecule consisting of
20 two heavy chains and two light chains and having a first binding
affinity for a human T-cell receptor capable of activating
killing and a second binding affinity for immunoglobulin, which
immunoglobulin binding affinity is for non-human immunoglobulin,
said fragment being either the F(ab')$_2$ fragment of the whole
25 molecule or a fragment of the whole molecule lacking only that
Fc region which is that of the heavy chain having a T-cell
binding affinity. •
28. A fragment according to Claim 27 of an antibody molecule as
defined in any of Claims 6 to 8.
30 29. A bi-specific antibody conjugate having a first binding
affinity for a T-cell receptor capable of activating killing and
a second binding affinity for target cells characterised in that
the conjugate comprises a bi-specific antibody molecule
consisting of two heavy chains and two light chains and having a
35 first binding affinity for a T-cell receptor capable of

activating killing and a second binding affinity for immunoglobulin linked to an antibody molecule having a binding affinity for target cells, or a fragment thereof retaining the binding affinities of the whole conjugate.

30. An antibody conjugate or fragment thereof which contains a bi-specific antibody molecule or fragment thereof as defined in any of Claims 1 to 28 in combination with an antibody molecule having a binding affinity for target cells, or a fragment thereof having such affinity.

31. An antibody conjugate according to Claim 29 or 30, in which the target cells are tumour cells.

32. A composition comprising an antibody molecule or conjugate or a fragment thereof according to any of Claims 1 to 31, together with a physiologically acceptable diluent or carrier.

33. A composition according to Claim 32, which additionally contains an antibody molecule having a binding affinity for target cells, or a fragment thereof having such affinity.

34. A kit which comprises in association an antibody molecule or fragment thereof according to any of Claims 1 to 28, and an antibody molecule having a binding affinity for target cells, or a fragment thereof having such affinity.

35. A composition according to Claim 33 or a kit according to Claim 34, in which the target cells are tumour cells.

36. A method of treatment of a neoplastic, viral or parasitic disease which comprises administering to a patient an antibody molecule or conjugate or a fragment thereof according to any of Claims 1 to 31.

37. The method according to Claim 36, in which a human patient is treated.

38. The method according to Claim 36 or 37, in which the disease is a neoplastic disease.

39. A process for the preparation of a bi-specific antibody molecule or fragment thereof according to any of Claims 1 to 28, which comprises culturing a fused hybridoma cell system which expresses said bi-specific antibody molecule in order to produce said molecule and thereafter, where appropriate, treating this to produce a fragment thereof.

40. A process for the preparation of a bi-specific antibody conjugate or fragment thereof according to Claim 29, 30 or 31, which comprises combining a bi-specific antibody molecule consisting of two heavy chains and two light chains and having a

05 first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin with an antibody molecule having a binding affinity for said target cells, or a fragment of either or both of said molecules, and thereafter, where appropriate, treating

10 the conjugate to produce a fragment thereof.

41. A bi-specific antibody molecule or conjugate or a fragment thereof whenever produced according to the process of Claim 40 or 41, or an obvious equivalent thereof.

42. A fused hybridoma cell system which expresses a bi-specific

15 antibody molecule according to any of Claims 1 to 25.

DATED this 17th day of October 1991.
NATIONAL RESEARCH DEVELOPMENT CORPORATION.

WATERMARK,
Patent & Trade Mark Attorneys
2nd Floor
"The Atrium"
290 Burwood Road
HAWTHORN   3122
Victoria
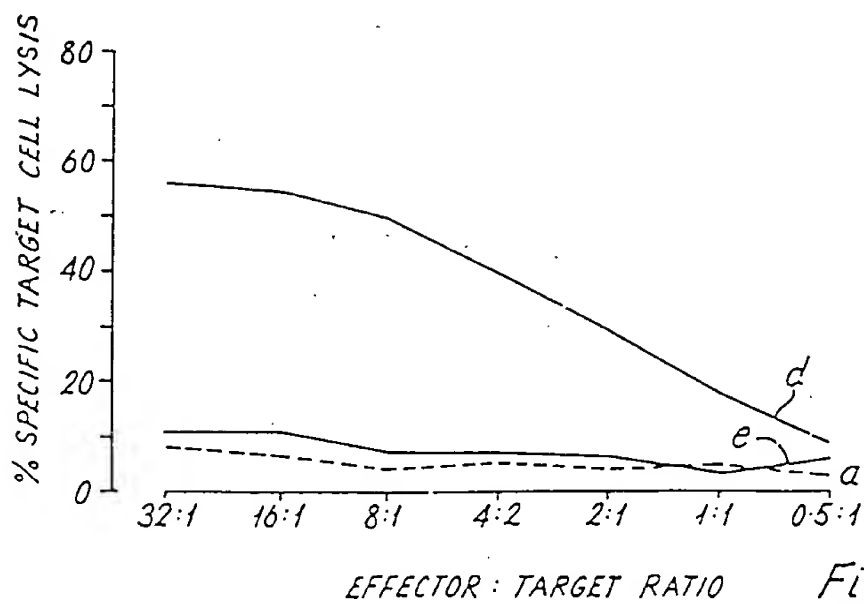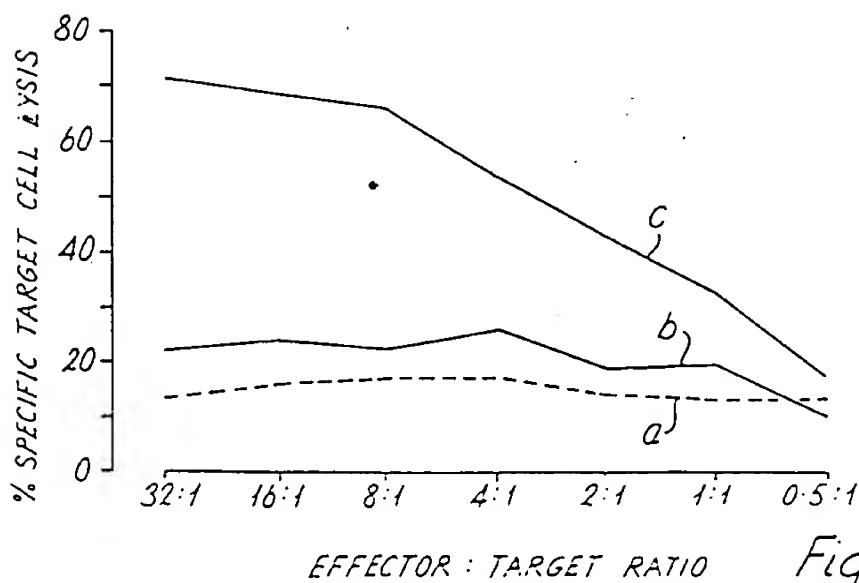Australia

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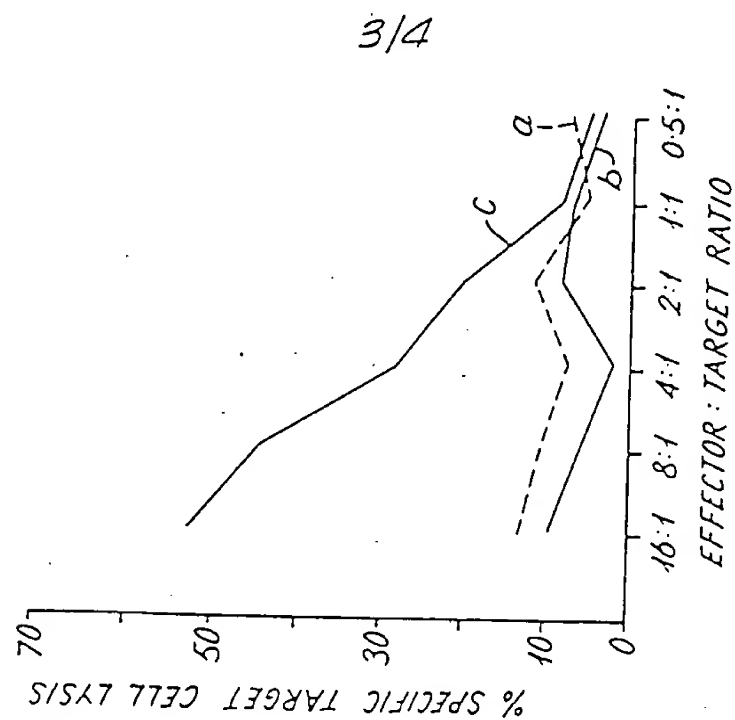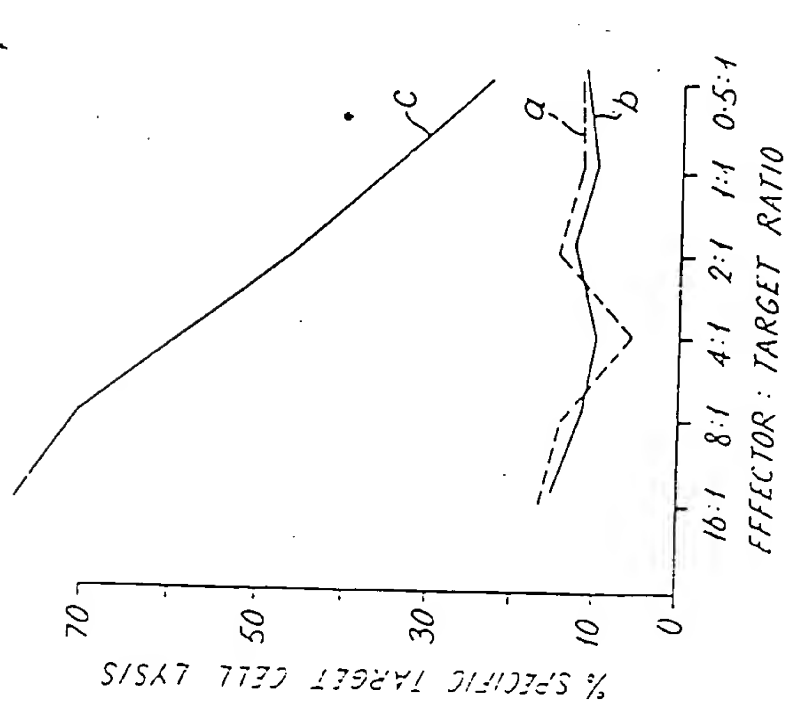
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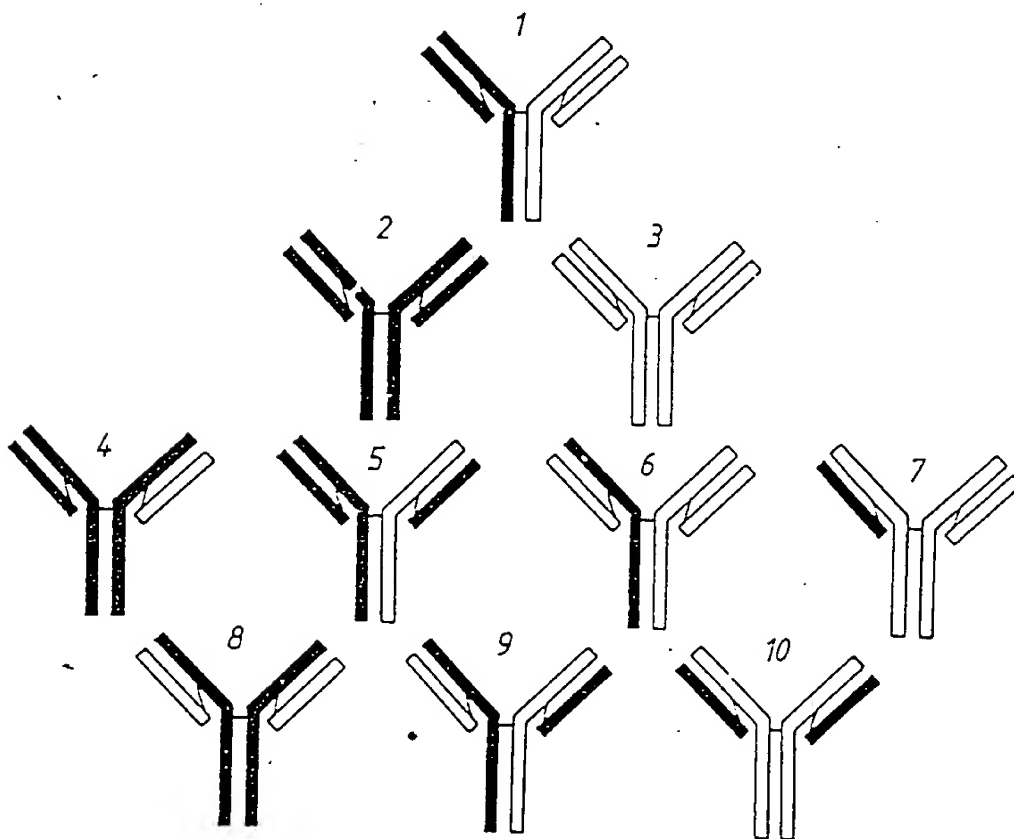
Fig. 1

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Fig. 2a

Fig. 2b

Fig. 3b

Fig. 3a

Fig. 3d

Fig. 3c